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## Molecular analysis of meningioma increases prognostic power: A methylation-based classification and grading system

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## Summary

### Background

The World Health Organization (WHO) classification of brain tumors describes 15 subtypes of meningioma. Nine of these are allotted to WHO grade I, and three each to grade II, and WHO grade III, respectively. Grading is ~~purely~~-based purely on histology, molecular markers are lacking. While the current classification and grading approach is of prognostic value, it harbors shortcomings such as ill-defined parameters for subtypes and grading criteria prone to arbitrary judgment.

### Methods

We investigated genome-wide DNA methylation patterns of 479 meningiomas to identify distinct methylation classes (MC) of meningioma. The MCs were further characterized by DNA copy-number analysis, mutational profiling and RNA sequencing. We validated our findings in an independent cohort of 140 meningiomas.

### Findings

DNA methylation profiling distinguished six distinct MCs associated with typical mutational, cytogenetic, and gene expression patterns. Meningioma MCs exhibit a more homogeneous clinical course and allow prognostication with significantly higher power than the current morphology-based WHO classification. Meningioma MCs more accurately identify patients at high risk of recurrence among tumors with WHO grade I histology, and patients at lower risk of recurrence among WHO grade II tumors. DNA methylation-based classification and grading reduces the number of meningioma subtypes from 15, as historically defined by histology, to six clinically relevant MCs, each with a characteristic molecular profile.

### Interpretation

DNA methylation-based meningioma classification captures biologically more homogenous groups and has a higher power for predicting tumor recurrence than the current WHO classification. The approach presented here is highly useful for stratifying meningioma patients for observation or post-surgery treatment groups. We consider epigenetic tumor classification highly relevant for future diagnosis and treatment of meningioma.

### Funding

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## Introduction

The meninges exert a protective function for the entire central nervous system (CNS). During development, their precursor cells merge from mesodermal structures and the neural crest, actively contributing to the differentiation of the brain<sup>1-3</sup>. However, meningeal cells may transform to initiate tumors, ~~which represent. These meningiomas are~~ the most frequent primary intra-cranial and spinal tumors<sup>4</sup>. While 80 % of meningiomas show benign clinical behaviour and can be cured by resection alone, about 20 % recur and need additional treatment such as repeated surgery, irradiation and systemic therapy<sup>4,5</sup>. Histopathological evaluation aims ~~at the to~~ identify ~~cases at risk for~~ recurrence. The histological ~~differentiation classification~~ into subtypes initially dates back to the 19<sup>th</sup> century. In ~~the~~ first internationally recognized classification ~~approach~~ in 1928, Bailey and Cushing distinguished ~~a~~ meningothelial, fibroblastic, and angiomatous subtypes<sup>6</sup>, and to this day, allocation to subtype is ~~purely~~ based solely on histological findings. The current WHO classification recognizes 15 subtypes and three grades of malignancy<sup>4</sup>, but some of the diagnostic criteria are vaguely defined and subject to a high inter-observer bias, indicating ~~the~~ need for more reliable biomarkers<sup>5,7</sup>.

For various other CNS tumors, molecular profiling has identified distinct subtypes with characteristic aberrations. Many of these correlate with prognosis or provide targets for treatment, and therefore support clinical decision making, e.g. epigenetic subgroups in medulloblastoma<sup>8-10</sup> and ependymoma<sup>11</sup>, or *isocitrate dehydrogenase (IDH)* status in diffuse glioma<sup>12-14</sup>. Recent studies identified *telomerase reverse transcriptase (TERT)* promoter mutations in a small subset of meningiomas to be associated with higher risk of recurrence and shorter time to progression<sup>15,16</sup>, and four large exome-sequencing efforts focusing on WHO grade I meningiomas have identified recurrently mutated genes beyond the long-known association with *NF2*<sup>17-20</sup>. Yet, these findings ~~only~~ cover only a fraction of meningiomas and have not all been ~~adequately~~ thoroughly tested for their prognostic relevance. In this study, we aimed at a comprehensive characterization of the entire molecular genetic landscape of meningioma in order to identify biologically and clinically relevant subgroups ~~that able to~~ refine the current classification scheme.

## Results

### *DNA methylation analysis identifies six distinct methylation classes of meningioma*

We generated genome-wide DNA methylation profiles from a discovery cohort of 497 meningiomas (Suppl. Fig. 1) along with 309 samples of other extra-axial skull tumors that histologically mimic meningioma variants, including solitary fibrous tumor/hemangiopericytoma, schwannoma, malignant peripheral nerve sheath tumors, chordoma, chondrosarcoma, fibrous dysplasia, and hemangioblastoma. Despite ~~shared~~ mesodermal origin, unsupervised clustering of DNA methylation data clearly segregated all meningiomas from ~~these~~ other skull tumors (Suppl. Fig 2). Unsupervised clustering of meningiomas alone revealed two major epigenetic groups (Groups A and B, Fig. 1A), with both groups further ~~subdividing-divisible~~ into four and two subgroups, respectively, termed “methylation classes” (MCs). Based on further molecular and clinical characteristics outlined below, the four MCs of Group A were designated MC benign 1 through 3 (MC ben-1, ben-2, ben-3) and MC intermediate A (MC int-A). The two MCs of Group B MC intermediate B (MC int-B), and malignant (MC mal).

There was an enrichment of grade I tumors among MC ben-1, MC ben-2, and MC ben-3, and an enrichment of WHO grade III tumors in MC mal, while WHO grade II tumors were scattered across all MCs. Analysis of 75 primary and matched recurrent tumors from 37 patients showed that association with Group A or B was stable upon recurrence (Fig. 1B), supporting further assessment of methylation profiling for diagnostic and prognostic implications.

### *MC predict clinical course with higher accuracy than WHO grading*

The wide spectrum of clinical behavior among WHO grade I and II meningiomas points towards the limited prognostic power of the current classification, particularly at the border between grade I and II. As a result, deciding on radiotherapy based on the current grading is heavily debated <sup>5</sup>. Thus, we correlated meningioma MCs with progression-free survival (PFS) to evaluate their potential for predicting outcome compared to WHO grading (Fig. 2A, B). We further combined MCs exhibiting virtually identical benign (MC ben-1, MC ben-2, MC ben-3) or intermediate (MC int-A, MC int-B) outcome into combined MCs (Fig. 2C). Classification by individual and combined MCs demonstrates more precise prognostication than by WHO grading (Fig. 2D, Brier prediction test,  $p < 0.01$ ). These findings were confirmed in 140 meningiomas from an independent validation cohort (Suppl. Fig 3A, B). We next focused on the ~~predictive~~ power of MCs within WHO grades and, particularly, patients divergently diagnosed by WHO grading and DNA methylation-based classification. Patients with WHO grade I meningiomas molecularly assigned to an intermediate MC experienced a less favorable clinical course than patients with WHO grade I meningiomas diagnosed solely based on histology. In fact, there

outcome was indistinguishable from that of patients with WHO grade II meningiomas (Fig. 3A). Likewise, patients with WHO grade II meningiomas molecularly assigned to a benign MC had a better outcome than the average outcome of patients with WHO grade II meningiomas. Consequently, stratification for MC is of higher value for prediction of PFS than WHO grading. Within the combined MCs, WHO grading confers limited additional information (Fig. 3B, Suppl. Table 2). However, combined MCs delineate subgroups with significantly distinct prognosis within all WHO grades (Fig. 3C), demonstrating the benefit of MC-based grading for patients and the potential to significantly reduce under- or overtreatment.

#### *Methylation classes are associated with distinct driver mutations and copy-number-alterations*

We next sequenced 304 meningiomas with sufficient material available using a custom hybrid-capture next-generation sequencing (NGS) panel dedicated to 40 genes previously reported to be mutant in meningioma (Suppl. Table 1), based on our recently established custom NGS approach for routine brain tumor diagnostics<sup>21</sup>. Known recurrent mutations (most frequently *NF2*, followed by *TRAF7* and *AKT1*) were significantly enriched in certain MCs (Suppl. Table 3, Fig. 4). Within Group A, *NF2* mutations were observed in 63 % of MC ben-1 tumors (accumulation of parameter in this MC  $p < 0.0001$ , Fisher's exact test). MC ben-2 contained the vast majority of meningiomas carrying *AKT1* (33 % in this subgroup;  $p < 0.0001$ ), *SMO* (7 %;  $p = 0.0002$ ), *KLF4* (15 %;  $p < 0.0001$ ), and *TRAF7* (49 %;  $p < 0.0001$ ) mutations and rarely *NF2* mutations. Only one *AKT1* and five *KLF4* mutations were detected outside this MC. MC ben-3 exhibited *NF2* mutations in 32 % and *PIK3CA* mutations in 11 % of tumors, representing the majority (5/7, 71 %) of *PIK3CA* mutations in the cohort. MC int-A carried *NF2* mutations in 53 %. Within Group B, MC int-B tumors harbored *NF2* mutations in 35 % and MC mal in 31 %. *SUFU* mutations were confined to Group B, with 5 % of MC int-B and 6 % MC mal tumors being mutated. Four out of five *TERT* promoter mutations mapped to the meningiomas in Group B ( $p = 0.005$  Fisher's exact test).

Annotation of copy-number-variations (CNV) revealed that MCs are associated with distinct cytogenetic aberrations (Fig. 4A, B): MC ben-1 was associated with deletions of 22q (95 %) but otherwise virtually no CNV. MC ben-2 presented with absence of recurrent CNVs. Typical for MC ben-3 were multiple chromosomal gains most frequently affecting chromosome 5 (47 %). MC int-A frequently exhibited losses on 1p (70 %) and 22q (84 %). In Group B, MC int-B frequently exhibited losses on 1p (89 %), 10 and 22 (89 %), all features also shared with MC malignant. However, in MC mal, a higher frequency of *CDKN2A* deletion occurred (70%).

Representative cases with sufficient material available of all MCs underwent RNA-sequencing in order to identify differentially upregulated genes and activated pathways (Suppl. Fig. 4). (In preparation...)

*Methylation classes and WHO subtypes, localization, and gender*

Examining the distribution of histological subtypes across MCs revealed which histological subtypes ~~comprise~~ the MCs ~~are composed of~~ and, conversely, to which MC the samples of a respective subtype are assigned (Fig. 5). The rare lymphoplasmacytic~~cell~~-rich meningiomas (WHO grade I) was not assessed due to the ~~overwhelming~~ dominance of constitutional (non-tumor) DNA in these samples. In general, two patterns were observed: Either a given MC was strongly associated with a small set of or even single subtypes, or samples of a MC or subtype, respectively, being widely spread across all variants. MC ben-1 comprised the majority of fibroblastic meningiomas and is also enriched for psammomatous meningioma. Fibroblastic meningiomas frequently harbor calcifications called psammoma bodies, and a high abundance of these calcifications defines psammomatous meningioma. The overexpression of SERPINF1 (Suppl. Fig. 4), which has been implicated in osteogenesis and calcification, in MC ben-1 might contribute to this histologically ~~detected~~ phenomenon. MC ben-2 was highly enriched for meningothelial meningiomas, and contained the vast majority of secretory meningiomas. MC ben-3 harboured cases from several subtypes but was particularly enriched for angiomatous meningiomas. This is in line with the overexpression of vessel-associated markers in samples of this MC (Suppl. Fig. 4). Transitional meningioma, a hybrid of meningothelial and fibroblastic histology, dissolved into several MCs, along with the samples of the rare microcystic, clear cell, chordoid, metaplastic and psammomatous subtype.

The two intermediate MCs were predominantly ~~constituted~~ ~~composed~~ of atypical meningiomas. However, a considerable fraction of atypical meningioma (n=31) fell into MC ben-1. These ~~observations~~ ~~majorly~~ contribute to the higher prognostic power of MC class over histology. Anaplastic meningiomas predominantly mapped to MC malignant. Of note, ~~the~~ six rhabdoid/papillary meningiomas, by definition WHO grade III, all ended up in one of the MC benign or intermediate meningioma groups. However, the number was too low to assess the statistical relevance of WHO grading and MC classification individually for rhabdoid/papillary meningioma.

Transitional meningioma WHO grade I was much more frequently assigned to an intermediate MC than fibroblastic or meningothelial meningioma, mostly to MC int-A. Atypical meningiomas assigned to a benign MC accumulated in MC ben-1.

The most frequent localizations for all subgroups were the frontal and ~~central~~ convexity, except for MC ben-2 (Fig 6). For the latter, basal localization was common, in line with the high occurrence of *AKT1* and *SMO* mutations in this MC, which are known to be enriched in this localization<sup>19,22</sup>. Interestingly, all MC mal cases were located ~~along~~ the convexity. In contrast, none of ~~the~~ basal tumors was allotted to MC mal, including four intraventricular and ten spinal meningiomas that all ~~localized~~ ~~went~~ to intermediate or benign MCs. ~~Gender and Age~~ distribution was equal throughout all

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MCs. ~~W~~with the exception of a predominance of male patient in MC mal, ~~while~~ all other MCs mainly comprised female patients (Fig 6).

## Discussion

The 15 subtypes of meningioma included in the current WHO classification have evolved over decades. The major aim of ~~introducing-including~~ this variety of subgroups was to cover the whole histological spectrum of meningioma and to avoid misclassification. For example, meningeal tumors with chordoid or rhabdoid cytology may initially raise ~~suspicion-the impression~~ of a chordoma or rhabdoid tumor ~~rather than but not point towards~~ a meningioma. Therefore, particular subtypes with ~~these-ambiguous~~ features were introduced into the classification in order to ~~draw attention to the morphologic diversity of highlight the existence of these cytological differentiations in~~ meningiomas. In addition, some cytological features have been reported to be associated with distinct outcomes. Although ~~often this was~~ based on small series, it prompted ~~the allocation allotment~~ of distinct WHO grades to specific meningioma subtypes. However, this approach has been increasingly questioned due to suboptimal inter-observer reproducibility<sup>7,23</sup>, most recently ~~reported~~ in a large Radiation Therapy Oncology Group (RTOG) meningioma trial <sup>7</sup> in which the authors expressed the urgent need for ~~more objective~~ molecular markers.

This resulted in an overall critical view of clinicians with respect to ~~the~~ current meningioma WHO classification and grading, which has been ~~reiterated-expressed~~ in the most recent published European Association of Neuro-oncology (EANO) guideline for the diagnosis and treatment of meningiomas<sup>5</sup>. ~~Accordingly, Re~~visiting meningioma diagnostics based on epigenetic profiling by defining MCs with enhanced predictive power will greatly improve ~~the~~ acceptance of meningioma classification and ~~facilitate more successfully guide clinical~~ decisions regarding postoperative treatment. An overview of ~~of~~ the molecular and clinical hallmarks of the six meningioma MCs is given in Figure 6.

### *Distinct methylation profiles suggest different development*

Beyond ~~the~~ identification of clinically relevant groups and the basis for a novel classification, our dataset might ~~give-provide~~ insight into the development of meningiomas. This has previously been shown for other entities ~~such as-F~~ the four variants of medulloblastoma, distinguishable by ~~their~~ DNA methylation patterns, ~~which~~ were shown to be determined by different precursor cell populations<sup>8,24-26</sup>, and exhibit very different clinical characteristics and therapy needs. Our data indicate that the spectrum of meningiomas is divided into two major epigenetically highly distinct Groups (A and B, Fig. 1). This strong separation suggests either the existence of distinct cells of origin or an underlying event with ~~a~~ major impact on genome-wide DNA methylation. The ~~distinctive very different~~ DNA methylation profiles of Groups A and B, despite the shared occurrence of *NF2* mutations, might suggest that meningiomas arise from two different precursor cell populations. Based on our own and published high-throughput sequencing data, there is no evidence for the existence of a single mutation being solely responsible for the separation of these two groups. However, we cannot fully exclude the

existence of alterations not readily detectable by these approaches, such as translocations or fusions, causing ~~the responsible~~ changes in the methylome. Moreover, the fact that patients with meningiomas clustering in Group A share a predominantly benign, with a small proportion exhibiting a semi-aggressive clinical course, and that patients with meningiomas of Group B follow a semi-aggressive to malignant clinical course, may further argue ~~towards-for~~ a distinct cell of origin with different intrinsic propensities for malignant transformation. However, analyses dissecting the full regulatory background of the tumor cells in comparison to arachnoidal cells, e.g. by ChIP-Sequencing, are needed to fully elucidate this.

#### *Methylation-based versus WHO subgrouping versus other molecular markers*

Extensive whole exome or -genome sequencing has provided a large body of information on the mutational landscape of meningioma<sup>17-20</sup>. Four distinct meningioma mutational subgroups have been proposed, defined by mutations either in *NF2*, *TRAF7*, the hedgehog pathway, or *POLR2A*<sup>18</sup>. However, such a model of meningioma development based on mutational analysis alone ~~currently~~ does not ~~currently~~ satisfy the clinical need for distinction between patients in need of adjuvant treatment or not. A major drawback is the lack of risk stratification among *NF2*-mutant cases ~~that~~~~which~~ can present with any clinical course. While the strong association of *AKT1*, *TRAF7/KLF4*, or *SMO* mutations with benign, or *TERT* promoter mutations with unfavorable course may allow for mutation-based risk assessment in these subgroups, the current inability to stratify *NF2*-mutated meningiomas for other mutational events associated with clinical outcome is a major obstacle for a classification and grading system based on mutational profiling alone.

~~Similarly~~~~Similarly~~, strong limitations apply to approaches based on copy-number-profiles: They leverage the accumulation of aberrations during progression but are not capable of predicting the ~~clinical~~ behavior ~~upfront~~. The current dataset attributes the highest prognostic power to methylome-based subgrouping, which proves to be superior to WHO classification (Fig. 2, 3). ~~However, with~~ an exclusively mutation-based ~~subgrouping for classification of~~ the full spectrum of meningioma ~~is~~ not yet in place.

#### *An integrated diagnosis for meningioma evaluation*

The WHO 2016 revision ~~of the classification of for~~ CNS tumors ~~classification~~ supports the concept of an integrated diagnosis. It relies on a multilayered approach combining data from histology, molecular genetic analyses, and clinical findings<sup>4,27,28</sup>. Adopting this WHO approach to the diagnosis of meningioma, the morphological layer corresponds to the current diagnostic standard, i.e. diagnosing the 15 WHO meningioma subtypes and grading according to the morphological scheme. In absence of

molecular analyses the morphological diagnosis should be suffixed with NOS (not otherwise specified), as agreed for parenchymal brain tumors without molecular workup<sup>4</sup>. The molecular diagnostic layer contains either DNA methylation or mutation analyses or both. With methylation analysis ~~performed~~, one of the six MCs can be diagnosed. Mutational data may only enable inferring the MC for a subset within the MC ben-2, e.g. for *AKT1* mutant cases, but not in each instance. If the data allow diagnosis ~~of a~~ MC, this results in a significantly ~~improved~~~~more powerful~~ prediction of the clinical course. This corresponds to the current approach ~~for in~~ other entities, e.g. ependymoma and medulloblastoma, ~~infer~~ which methylation data has proven to be more relevant than histological grading<sup>4,11</sup>. Based on the data presented here, the integrated diagnosis of meningioma will ~~not only also highlight identify~~ the ~~prognostically relevant~~ MC ~~with regard to prognosis~~ but ~~also provide in addition refer to~~ the morphological subtype identified ~~on in~~ histological examination.

Collectively, the ~~accompanying~~ dataset and ~~proposed accompanying~~ classification scheme ~~proposed here~~ advances meningioma diagnostics from ~~the traditional histopathological approach histology into~~ an integrated profiling with higher accuracy ~~of in~~ risk assessment for individual patients.

#### Author contributions

FS and AvD conceived the project, coordinated data generation, and wrote the manuscript with input from all co-authors. FS, D Schrimpf, D Stichel, DTWJ, SS, D Sturm, M Sill, VH, LC and SMP designed methylation experiments and analyzed DNA methylation data. FS, D Schrimpf, D Stichel, LC and KO analyzed RNA sequencing data. FS, D Schrimpf and TH analyzed survival data. FS, D Schrimpf, D Stichel, SS, DR, CK, DC, KH, AK, AW analyzed DNA sequencing data. M Schick and MBH performed array experiments. FS, DR, CK, DC, KH, AK, AW, PB, K Kurian, AFO, CM, AK, JS, EJR, VPC, WP, MM, and AvD performed histological evaluation. HGW, ASB, PB, HE, K Kurian, AFO, CM, CJ, MSR, RK, M Simon, AB, KL, AK, JS, VPC, SB, M Platten, DH, AU, WP, WW, MM, M Preusser, CHM, and MW collected and interpreted clinical data and/or compiled respective tissue collections.

## Materials and Methods

### *Samples*

Samples with clinical data were retrospectively collected from the Dept. of Neuropathology Heidelberg, Germany (local and referral cases), Dept. of Neurosurgery Heidelberg and the FORAMEN network, the Dept. of Neurology and Neuropathology, Zürich, Switzerland, and the Neurological Institute (Edinger Institute) Frankfurt/Main, Germany. Additional samples without survival annotation were included from the Dept. of Neuropathology Berlin, Bonn, Magdeburg, Münster, Tübingen (all Germany), and Bristol (UK). The validation cohort was provided by the Medical University of Vienna.

### *Methylation analysis, copy-number analysis*

Illumina 450k Human BeadChip (discovery cohort) and 850k EPIC (validation cohort) analysis were performed as previously described (ref). Unsupervised clustering for the discovery and validation cohort was performed based on XYZ (Euclidian ward SD 0.2). Copy-number aberrations were inferred from methylation array data (Ref VH).

### *Copy number analysis in MCs*

Damian

### *Panel and RNA sequencing*

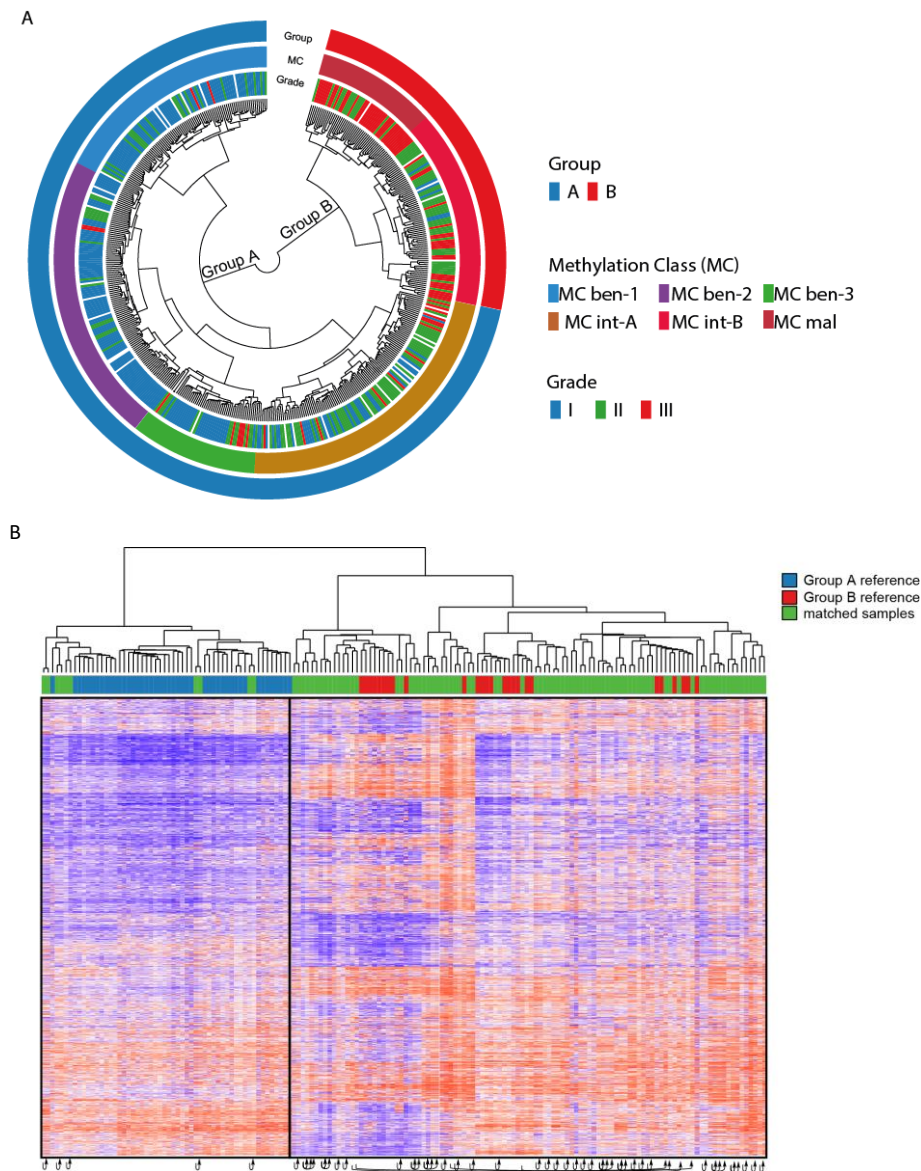
Panel sequencing for genes reported to be mutant in meningioma (Suppl Table 2) was performed applying a custom hybrid-capture approach (Agilent) as described before (Ref). RNA libraries were generated with TruSeq RNA Access (Illumina). Sequencing was performed on a NextSeq 500 (Illumina).

### *Expression analysis based on RNA-seq data*

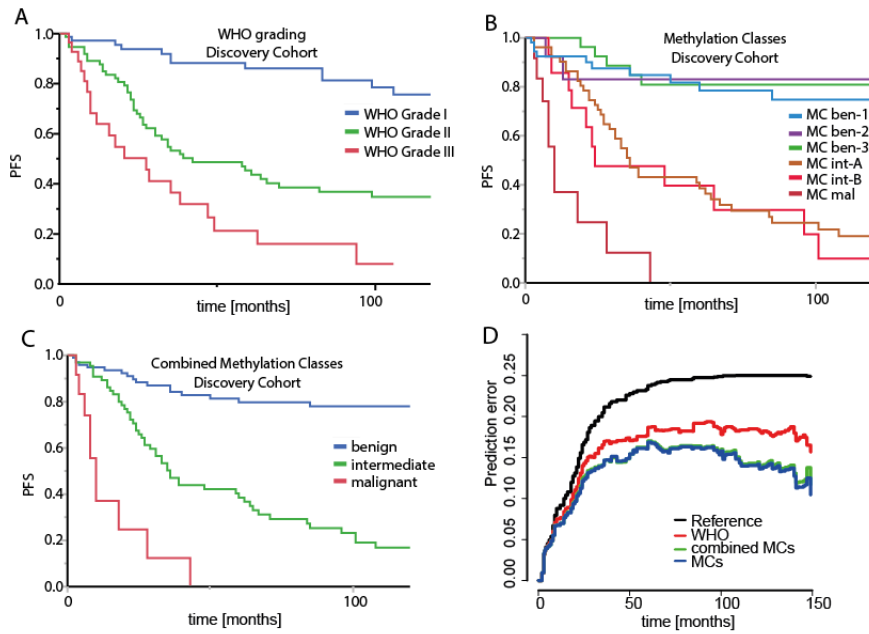
Konstantin

### *Statistical analysis of clinical parameters*

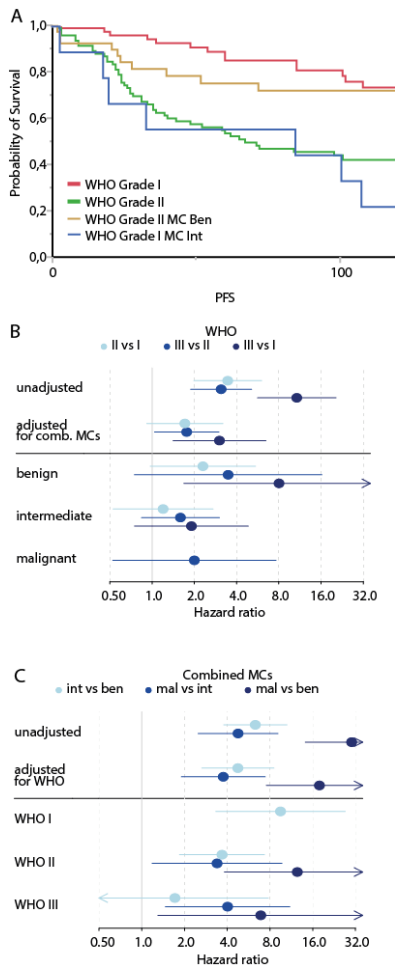
Distribution of survival times was estimated by the method of Kaplan and Meier and compared between groups with the log-rank test. Hazard ratios including 95% confidence intervals based on Cox regression models were calculated. For the multivariable Cox regression model, imputations of missing covariate values was done applying the multivariate imputations using chained equations (mice) algorithm with 100 imputation runs. Hazard ratio for age is given per 10 year increment. Prediction error curves based on the Brier score were computed. Integrated Brier score was tested between risk stratifications using 1000 bootstrap samples. P-values below 0.05 were considered statistically significant. Analyses were performed with statistical software R 3.3. Details and referecens are given in the supplemental information.



**Figure 1** Unsupervised clustering of methylation data of 497 meningioma samples (A). Unsupervised clustering of matched primary and recurrent samples (matched primary/recurrent samples of identical patient identified by arrows) combined with reference samples from group A and B shows that no shift between groups occurs upon recurrence (B).

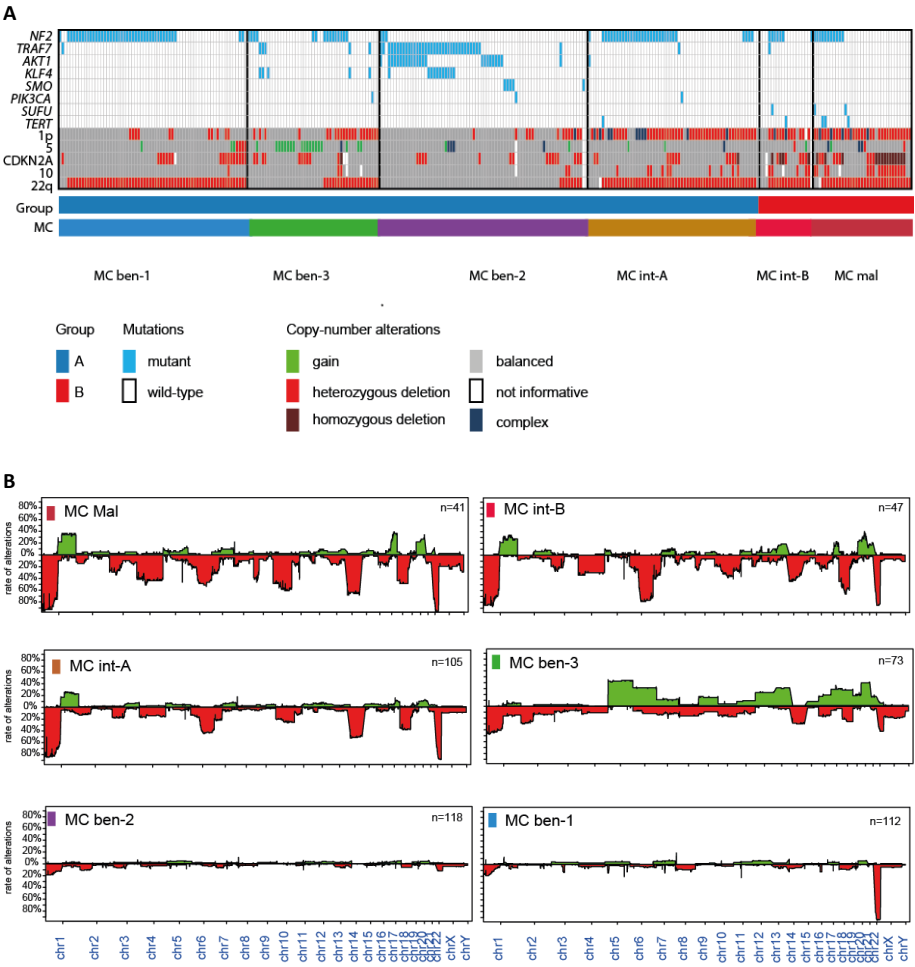


**Figure 2** Progression free survival (PFS) of 228 case with clinical data stratified for WHO grade (A), methylation class (B), combined methylation classes (C). Brier prediction plot calculated for the models A-C (D, WHO vs combined MCs  $p=0.0138$ ,  $0.0096$ ,  $0.0062$  for 5, 10 and 12 years, respectively).

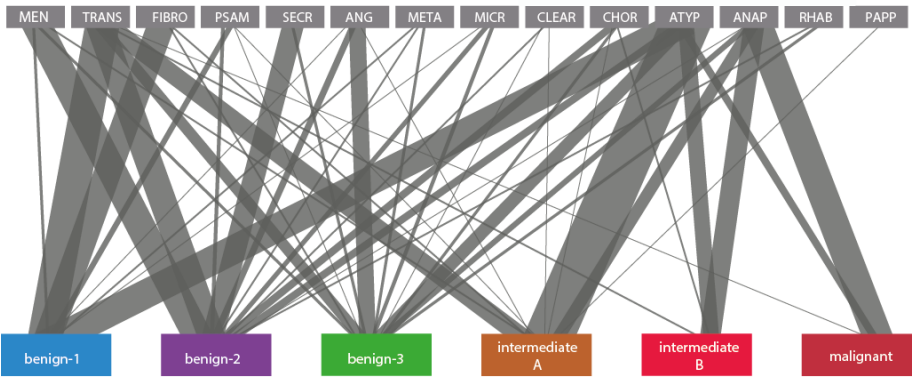


**Figure 3** Comparison of WHO grading and methylation-based risk prediction: WHO grade I cases allotted to an intermediate methylation class show PFS similar to the average grade II tumors. In turn, WHO grade II cases assigned to a benign methylation class have longer PFS than the average WHO grade II cases (A). Hazard ratio (including 95% confidence intervals) forest plot for WHO grading, overall and stratified for combined methylation classes (B). Hazard ratio forest plot for combined methylation classes, overall and stratified for WHO grading (C). While sub-stratification for WHO grade among MCs is of limited additional value (B), MCs stratify for distinct PFS within WHO grades (C).

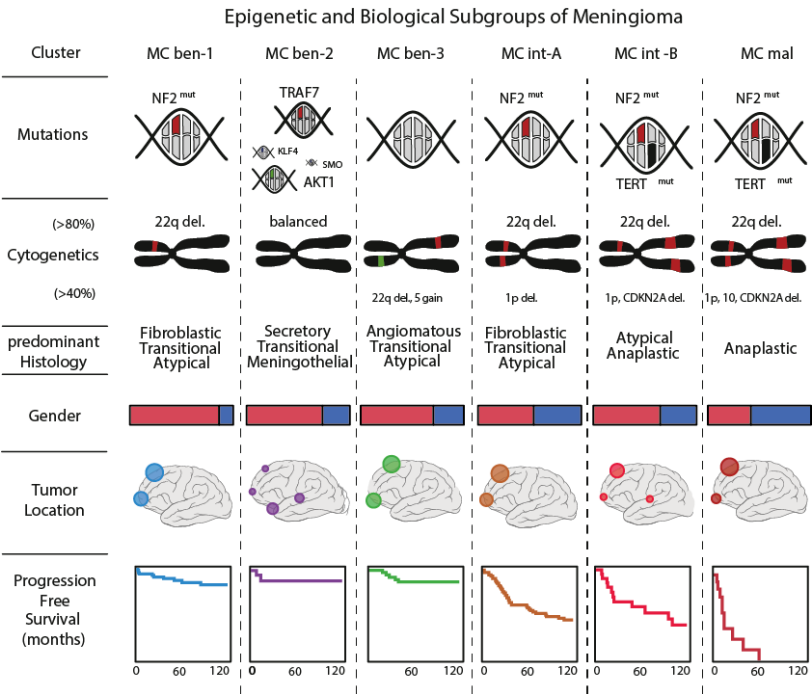




**Figure 4** Distribution of mutations across sample that underwent panel-sequencing (304) stratified for MCs (A). Copy number variations across all samples that underwent 450k analysis (497) the MCs (B).

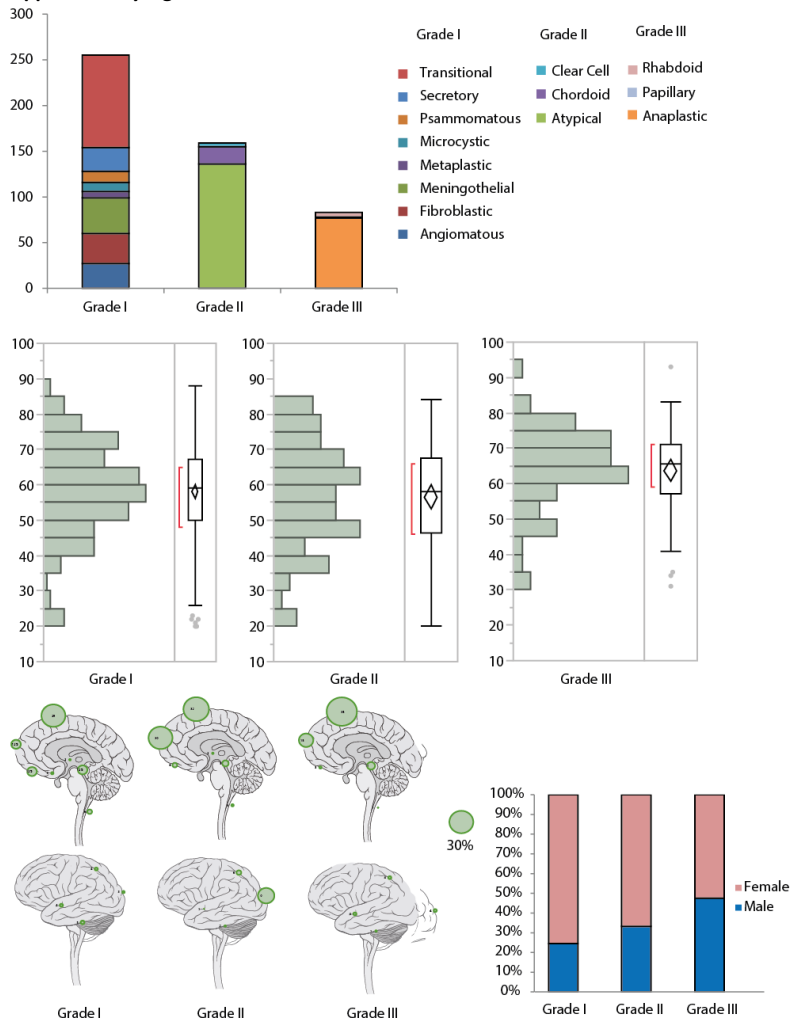


**Figure 5** Association of histological subtypes and MCs



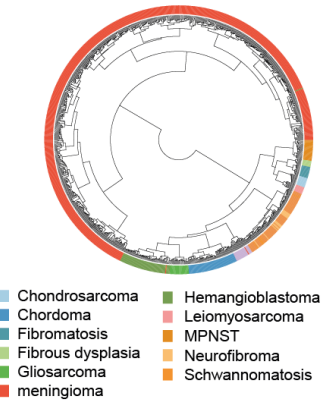
**Figure 6** Schematic overview over the six identified MCs and their molecular and clinical characteristics

Supplementary Figure 1

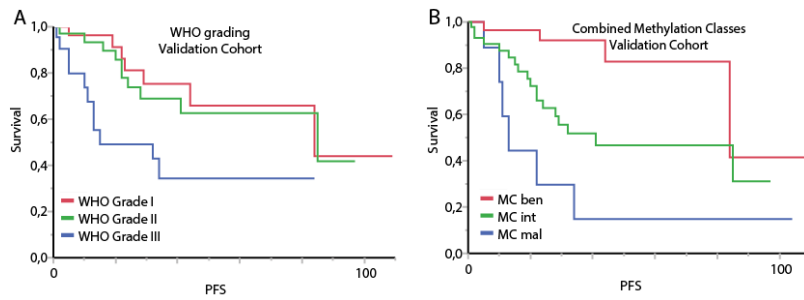


Supplementary Figure 2

A



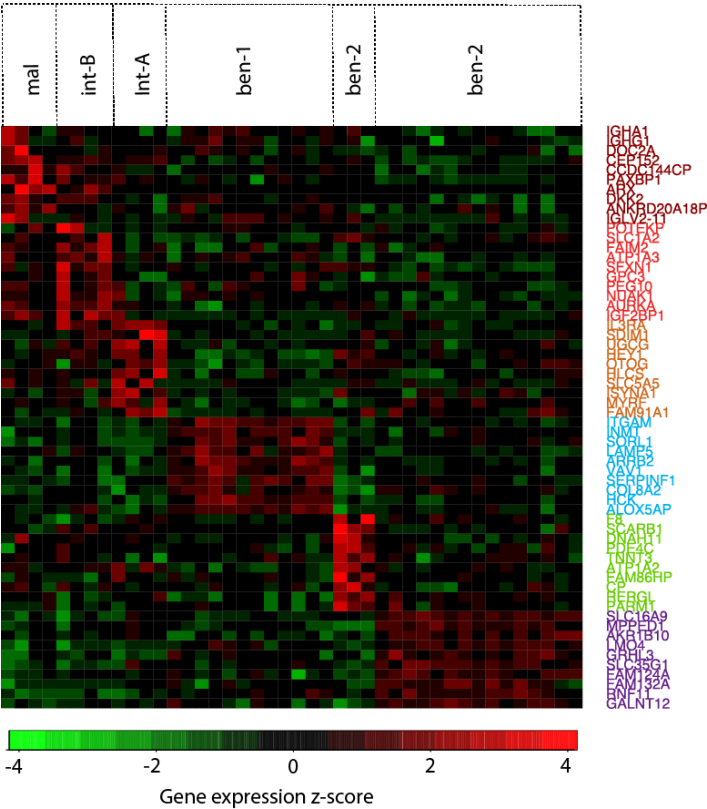
**Supplementary Figure 3**



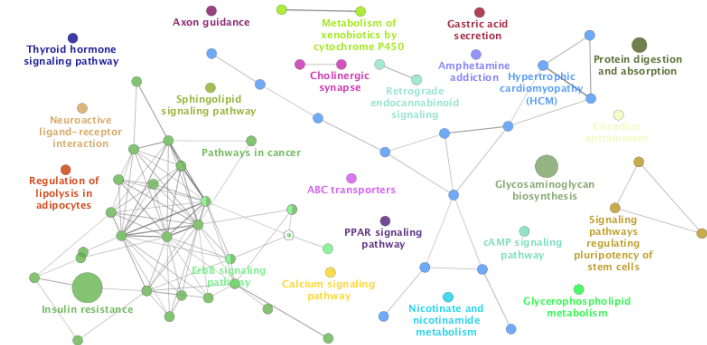
Validation cohort stratified for WHO grade (A) and combined methylation classes (B).

Supplementary Figure 4

A



B



Most differentially expressed genes in the six MCs (A) and ClueGo based on KEGG source data for MC ben-2 (B) (further MCs following)

Supplementary Table 1

In preparation

Supplementary Tables 2

Multivariable analysis

	coef	HR = exp(coef)	95% CI	p-value
age	-0.23	0.80	[0.66, 0.96]	0.02
male sex	0.19	1.20	[0.71, 2.04]	0.49
SimpsonGrade2	-0.02	0.98	[0.55, 1.75]	0.95
SimpsonGrade3	0.07	1.07	[0.49, 2.35]	0.86
SimpsonGrade4-5	0.20	1.22	[0.53, 2.84]	0.64
convexity	0.14	1.15	[0.61, 2.18]	0.66
frontal	0.34	1.40	[0.60, 3.26]	0.43
other	0.18	1.19	[0.25, 5.74]	0.83
posterior fossa	-0.01	0.99	[0.35, 2.80]	0.98
ClinSubgroupintermediate	1.56	4.78	[2.48, 9.21]	< 0.0001
ClinSubgroupmalignant	2.96	19.30	[7.91, 47.07]	< 0.0001
center HD	-0.60	0.55	[0.27, 1.12]	0.10
center Zurich	-0.30	0.74	[0.34, 1.62]	0.45
WHOII	0.31	1.37	[0.69, 2.71]	0.37
WHOIII	1.01	2.75	[1.22, 6.20]	0.01

	coef	HR = exp(coef)	95% CI	p-value
age	-0.22	0.80	[0.66, 0.97]	0.03
male sex	0.23	1.26	[0.74, 2.12]	0.39
Simpson Grade2	0.04	1.04	[0.58, 1.87]	0.90
Simpson Grade3	0.27	1.31	[0.60, 2.88]	0.50
Simpson Grade4-5	0.37	1.45	[0.63, 3.37]	0.39
convexity	0.25	1.28	[0.67, 2.44]	0.45
frontal	0.52	1.69	[0.73, 3.91]	0.22
other	0.09	1.09	[0.23, 5.24]	0.91
posterior fossa	-0.04	0.96	[0.34, 2.69]	0.94
ClinSubgroupintermediate	1.84	6.29	[3.48, 11.39]	< 0.0001
ClinSubgroupmalignant	3.41	30.38	[13.49, 68.38]	< 0.0001
centerHD	-0.42	0.66	[0.34, 1.29]	0.22
centerZurich	-0.04	0.96	[0.46, 1.99]	0.91



Supplementary Table 3

Distribution of Mutations across MCs

	PIK3CA	AKT1	KLF4	TRAF7	NF2	SMO	SUFU	TERT	n
Ben-2	1 1%	24 32%	11 15%	36 49%	0 0%	5 7%	0 0%	0 0%	74
Ben-1	0 0%	0 0%	0 0%	1 1%	42 63%	0 0%	0 0%	0 0%	67
Ben-3	5 11%	0 0%	5 11%	5 11%	15 32%	0 0%	0 0%	0 0%	47
Int-A	1 2%	1 2%	0 0%	1 2%	32 53%	0 0%	0 0%	1 3%	60
Int-B	0 0%	0 0%	0 0%	1 5%	7 35%	0 0%	1 5%	1 5%	20
Mal	0 0%	0 0%	0 0%	0 0%	11 31%	0 0%	2 6%	3 9%	35

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